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Effect of Serum Progesterone Levels at Estrus on Conception in Graded Murrah Buffaloes under Field Conditions

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Abstract

The present study was taken up to access the effect of serum progesterone at estrus on conception in Graded Murrah buffaloes under field conditions for which 70 parous buffaloes maintained under village system of rearing, free from apparent pathological abnormalities of the reproductive tract presented in the first postpartum AI were utilized. Serum samples were collected on day 0 and 10 for estimation of progesterone by RIA. The animals were re-examined using ultrasound on day 10 following insemination for determining the CL size and day 30 for pregnancy diagnosis which is confirmed per rectally by day 60 post AI. The mean values of serum progesterone at AI and on day 10 post AI were calculated and the relation was analyzed. Serum progesterone concentration at the time of AI and pregnancy status was negatively correlated indicating that when progesterone level drops < 0.3 ng/ml (basal level) at the time of AI, the chances of the animal becoming pregnant were more.

Keywords: Serum progesterone, Murrah buffalo, transrectal ultrasonography

In some studies on dairy cattle, it was speculated that POF diameter is important for subsequent development of CL and conception. A large POF may generate a large CL that will secrete more progesterone and thereby have a positive effect on pregnancy rates (Busch *et al*., 2008 and Binelli *et al*., 2009). On the contrary other authors have reported absence of such a correlation (Colazo *et al*., 2009) or negative correlation (Lynch *et al*., 2010) between POF diameter and pregnancy success. Review of literature revealed paucity of critically derived information on follicular and luteal structures in relation to pregnancy in

buffaloes maintained under field conditions, indicating that no detailed study has been undertaken in these areas. Further, there is no information regarding the percentage of buffaloes reported for AI by the owners at right time so as to achieve optimum fertility.

Materials and Methods

The present study was conducted at TVCC, NTR CVSc, Gannavaram from the month of Oct. 2014 to May, 2015 using 70 buffaloes maintained under village system of rearing free from apparent pathological abnormalities of the reproductive tract were selected and used for this study. Once the estrus was confirmed, the estrus intensity was studied. Based on the estrus symptoms, the intensity of estrus was classified as weak, intermediate and intense, on the basis of score card devised by Rao and Rao (1981) with slight modification. Blood samples were obtained from 40 buffalo cows at the of time of AI and on day 10 via venipuncture (jugular vein) into 5 ml vacutainer tubes to determine serum progesterone concentration. Blood was allowed to coagulate at room temperature, stored at 4° C for 24 h, and centrifuged at 1200 RPM for 30 min. Serum was collected and stored at - 20°C until Radioimmunoassay (RIA). Serum progesterone was determined using a commercial solid-phase RIA kit containing anti progesterone antibody-coated tubes, and I125-labeled progesterone (Immunotech, Beckman Coulter, France) (Pfeifer *et al*., 2009).

Estimation of progesterone in serum samples was done at the National Institute of Animal Nutrition and Physiology (NIANP), Bengalure (A unit of Indian Council of Agricultural Research) using Packard Gamma Multiwell counters (USA). The serum progesterone concentration was expressed in nanograms per milliliter (ng/ml). Transrectal ultrasonographic monitoring (Real time B mode) of ovarian follicles with 7.5 MHz linear array rectal transducer (PROSOUND, ALOKA, JAPAN). The animals are re-examined using ultrasound on day 10 following insemination for the presence of CL which was measured in a similar manner as the follicles. All the buffaloes were examined transrectally by using B mode real time ultrasonography for pregnancy diagnosis at day 30 after breeding which was confirmed per rectally on day 60. All statistical analysis was performed using the SPSS (20.0) system for Windows.

Results and Discussion

POF size: During the present study, out of 70 she buffaloes scanned for POF size, 15, 35 and 20 buffaloes recorded SPF, MPF and LPF, respectively and mean of SPF, MPF and LPF were recorded as 21, 50, and 29 per cent. The overall mean diameter of the POF in pregnant group (12.71±0.39) was slightly

larger than nonpregnant group (11.91±0.42) buffaloes but the difference was not statistically significant (P>0.05) (Table 1). Out of 70 she buffaloes scanned for POF size , 15, 35 and 20 buffaloes recorded as SPF (<9 to ≤12 mm), MPF (>12 to ≤14mm) and LPF(>14 mm), with group means of 10.53±0.19, 13.36±0.09 and 15.4±0.13 mm, respectively, with a mean diameter of 12.31±0.29 ranging from 9 to 16 mm.

These findings are concurring, with the findings of Baruselli (1997) in Murrah buffaloes (13.3±1.8 mm) and Presicce *et al.* (2005) in primiparous and pluriparous Mediterrean Italian buffaloes during postpartum estrus with a mean POF size of 13.5±0.8 mm. Similar findings were also reported, in Iraqi buffaloes by Azawi *et al.* (2009), who reported the POF size, on the right and left ovaries as 10.78 and 11.24±2.15 mm, respectively. Barkawi *et al.* (2009) reported the maximum diameter of POF as 14-15 mm in Egyptian buffaloes. Yindee *et al.* (2011) in swamp buffaloes revealed the presence of ovulatory follicles with a mean diameter of 13.5±0.52 to 14.17±1.08 mm during the post-partum ovulations. Contrary to the present findings, Derar *et al*. (2012) reported the POF size as 9.8±0.32 mm in Egyptian buffaloes which was much lower than the present observations. However, Taneja *et al.* (1995) and Neglia *et al*. (2007) reported the ovulatory follicle size as 15.3±0.03 mm in non-lactating Murrah buffaloes and in Italian Mediterranean buffaloes (16.9±0.16 vs 14.9±0.25 mm), respectively which was greater than the diameter of pre ovulatory follicle size observed in the present study. These differences could be due to location, season (Badinga *et al*., 1994), feeding and managemental practices adopted under farm and field conditions. The progesterone concentration on day 0 was significantly different among the three groups of POF viz., SPF, MPF and LPF with 2.92±0.08, 3.03±0.70 and 3.39±0.10 ng/ml, respectively.

The maximum POF diameter was positively correlated with the estrus intensity which is said to be highly significant (P< 0.01) for the intensity of estrus whereas the POF diameter is negatively correlated with progesterone concentration on day 0. This effect was statistically highly significant (Table 2). The ultrasonic determination of diameter of CL on day 10 post AI was carried out and grouped as >12 to ≤15, >15 to ≤18 and >18 mm with mean values of 13.77±0.23, 17.06±0.21 and 20.16±0.40 mm, respectively. The overall mean CL size was 15.60±0.49 mm having a range of 10-21 mm. The overall mean progesterone concentration on day 10 was 3.04±0.06 ng/ml. The difference in CL size between pregnant and nonpregnant buffaloes was significant (17.83 vs 14.9 mm), (P<0.05) (Table 1). The size of CL was significantly different (P<0.05) among the three groups of POF. The CL size in SPF, MPF and LPF

was 14.09±0.61, 16.00±0.48 and 18.90±0.60 mm, respectively. The diameter of POF at AI positively correlated with the diameter of CL at day 10 after AI which is statistically highly significant (r = 0.857, P<0.01).

The progesterone concentrations to the different sizes of CL were 2.92±0.08, 3.03±0.70 and 3.39±0.10 ng/ml, respectively with significant difference. The results suggest that the size of the CL was influenced by the size of POF at the time of estrus (Table. 2). The ovulation rate was significantly lower in buffaloes that had POF size 9 to ≤12 mm (LPF) compared to other two groups whereas pregnancy rate was highest (51.43 per cent) in MPF group. The maximum diameter of the POF at the time of AI was positively correlated with the conception rate but it is statistically nonsignificant (r = 0.906, P>0.05) (Table 2).

Serum progesterone on day 0: Serum progesterone on the day of estrus was analyzed in 40 buffaloes and with a mean value of 0.67±0.12 ng/ml. The data for the buffaloes were classified into three categories namely, buffaloes that had basal level of progesterone (<0.3 ng/ml) at the time of first AI, between 0.35 to 1 ng/ml and >1ng/ml with mean values of 0.23±0.10, 0.89±0.05 and 1.20±0.17 ng/ml, respectively. The ovulation rate for the buffaloes that had < 0.3ng/ml and >0.3 to 1 ng/ml was 100 per cent and 90.90 per cent respectively, indicating that all the buffaloes that had<1ng/ml of progesterone at the time of first AI were ovulated. The ovulation rate was significantly lower (P<0.01) in buffaloes that had serum progesterone value of > 1ng/ml compared to buffaloes that had serum progesterone concentration of < 0.3ng/ml.

The pregnancy rate was higher (65.90 per cent) in buffaloes that had < 0.3ng/ml progesterone concentration compared to buffaloes that had progesterone concentration between 0.3–1ng/ml (38.88 per cent) and >1 ng/ml (0 per cent) at the time of AI. The mean values of serum progesterone in pregnant and nonpregnant buffaloes were 0.89±0.13 and 1.42±0.23 ng/ml, respectively. The difference was not statistically significant (P>0.05) (Table 1). In the study, the mean progesterone level at the time of AI or estrus (Day 0) was found to be 0.67±0.12 ng/ml which is in close agreement with several earlier studies which reported that blood progesterone concentration, in blood plasma of Murrah buffaloes at estrus was 0.1ng/ml which elevated to a peak of 3.6ng/ml on day 13 (Batra *et al*., 1979). It continued to increase in animals that conceived but dropped to 0.6ng/ml on 3 days before the next estrus in those that failed to conceive.

Similar finding was also reported by (Arora and Pandey, 1982) with basal levels of (0.1-0.3ng/ml) during estrus and remained close to 1ng/ml for the next 3-4

days. Takkar *et al.* (1982) reported the progesterone levels as 0.360±0.062 and 0.334 ± 0.066 ng/ml on the day of estrus in buffalo heifers and buffalo cows, respectively.

The values were around 1ng/ml till day 6 followed by a gradual increase to a peak average value of 4.888±0.399 and 5.119±0.415 ng/ml on day 15 of the cycle in heifers and cows respectively. Mondal *et al.* (2010) reported the plasma progesterone concentration as 0.30±0.06 to 1.94±0.03 ng/ml during the estrus cycle in buffaloes. Plasma levels which were lowest during peri estrus phase increased to 0.47±0.70 ng/ml during early luteal phase and then further to 1.94±0.30 ng/ml during the mid-luteal phase. Peak progesterone values of 4–5.1 ng/ml have been recorded about 15 days after estrus (Bachlaus *et al.*, 1979; Arora and Pandey, 1982 and Takkar *et al.*, 1982). In the present investigation, the progesterone concentration at the time of AI or estrus (day 0) was significantly different (P<0.01) among the three groups of POF Viz., SPF, MPF and LPF with 0.92±0.20, 0.76±0.17 and 0.40±0.14 ng/ml, respectively. More number of animals showed intermediate estrus (72.85 per cent; n = 51) followed by weak (17.14 per cent; n = 12) and Intense (10 per cent; n = 7) estrus signs.

The ovulation rates were higher in buffaloes that exhibited intermediate estrus whereas pregnancy rates were higher in intense estrus group however, statistical analysis of the data failed to establish any significant difference (P>0.05) between these groups. The ovulation rate for the buffaloes that had <0.3ng/ml and >0.3 to 1 ng/ml was 100 per cent and 90.90 per cent respectively, indicating that all the buffaloes that had<1ng/ml of progesterone at the time of first AI have ovulated. The ovulation rate was significantly lower (P<0.01) in buffaloes that had serum progesterone value of >1ng/ml compared to buffaloes that had serum progesterone concentration of <0.3ng/ml. Inadequate luteolysis prior to estrus might have resulted in higher circulating progesterone level near AI which subsequently resulted in anovulation (Wiltbank *et al.*, 2002).

The pregnancy rate was higher (65.9 per cent) in buffaloes that had < 0.3ng/ml progesterone concentration compared to buffaloes that had progesterone concentration between 0.3-1ng/ml (38.88 per cent) and >1 ng/ml (0 per cent) at the time of AI. The mean values of serum progesterone in pregnant and nonpregnant buffaloes were 0.895±0.134 and 1.429±0.235 ng/ml respectively. In this study, more number of buffaloes became pregnant, when the progesterone concentrations at the time of AI were below suprabasal level and the pregnancy reduced when the progesterone level is between suprabasal and 1 ng/ml. None of the cows become pregnant when the progesterone level

was more than 1 ng/ml. The present result concurs with the findings of De Silva *et al*. (1981) who reported that higher progesterone level at the time of estrus might affected sperm and ovum transport, as well as the fertilization process and subsequent embryo passage to the uterus. The present result is also in agreement with the findings of Duchens *et al.* (1995) who reported that supra-basal progesterone level will delay the ovulation and lead to retention of graafian follicle for an extended period and cause damage of the oocyte to such an extent that even inseminating close to the time of ovulation may not ensure fertilization.

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